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Pathogenicity of *Listeria monocytogenes* Scott A After Oral and Oculonasal Challenges of Day-Old Turkey Poults

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SUMMARY. *Listeria monocytogenes* is a ubiquitous, environmental pathogen that has contaminated poultry ready-to-eat products resulting in large-scale recalls. Research is needed to determine the source of product and processing plant contamination with *L. monocytogenes*. The purpose of this study was to compare the oral and oculonasal routes of infection on the pathogenicity of *L. monocytogenes* in turkey poults under different housing conditions. One-day-old turkey poults were challenged by either route with the Scott A strain of *L. monocytogenes* and placed either in paper-lined battery-brooder cages for 1 wk or in floor pens on fresh pine-shaving litter. On day 7, birds challenged in battery cages were transferred to floor pens. Challenge by the oculonasal route resulted in higher mortality ($P = 0.05$) and lower body weights ($P < 0.0001$) compared with both nonchallenged controls and those challenged by the oral route. Birds contained in battery cages for 1 wk had higher mortality ($P = 0.002$) and higher body weights ($P < 0.0001$) compared with floor-pen–reared birds. Using direct plating, the challenge strain was isolated from the gall bladder, brain, and knee joint of only one dead poult challenged by the oculonasal route. These results suggest that day-old turkey poults may be more susceptible to an oculonasal challenge with *L. monocytogenes* than to an oral challenge and that containment in battery cages for the first week increased contact exposure to the challenge.

RESUMEN. Patogenicidad de *Listeria monocytogenes* Scott A luego del desafío oral y oculonasal de pavitos de un día de edad. La *Listeria monocytogenes* es un patógeno ambiental ubicuo que ha contaminado productos avícolas listos para consumir resultando en retiros masivos del mercado. Se requiere investigación para determinar el origen de la contaminación con *L. monocytogenes* de productos y de la planta de procesamiento. El propósito de este estudio fue comparar el efecto de las rutas de infección oral y óculonasal en la patogenicidad de *L. monocytogenes* en pavos jóvenes bajo diferentes condiciones de crianza. Se desafiaron pavitos de un día de edad con *L. monocytogenes* por alguna de las dos rutas y se colocaron en jaulas de crianza con papel en el piso por una semana o se colocaron en corrales con cama de viruta de pino. Al día siete, las aves desafiadas en las jaulas se transfirieron a los corrales en piso. El desafío por la vía óculonasal resultó en mayor mortalidad ($P = 0.05$) y en pesos corporales mas bajos ($P < 0.0001$), en comparación con los controles no desafiados y las aves desafiadas por la vía oral. Las aves mantenidas en las jaulas por una semana presentaron una mayor mortalidad ($P = 0.002$) y mayor peso corporal ($P < 0.0001$) que las aves criadas en el piso. Utilizando siembra directa, se aisló la cepa de desafío de la vesícula biliar, cerebro y articulación de la rodilla de sólo una de las aves muertas desafiada por la vía óculonasal. Estos resultados sugieren que los pavitos de un día de edad pueden ser más susceptibles a un desafío óculonasal con *L. monocytogenes* que a un desafío oral y que el confinamiento en jaulas durante la primera semana incrementó la exposición por contacto al desafío.

Key words: turkeys, *Listeria monocytogenes*, pathogenesis

Abbreviations: Baso = basophils; CFU = colony forming units; EDTA = ethylenediaminetetraacetic acid; Eos = eosinophils; FAC = ferric ammonium citrate; Het = heterophils; Lym = lymphocytes; Mono = monocytes; plt = platelets; TPB = tryptose phosphate broth; UVM = University of Vermont medium; WBC = white blood cells

Listeria monocytogenes is a bacterial pathogen of food-safety importance to the poultry industry. Its ability to contaminate poultry products and processing plants is generally considered to be because of its widespread environmental presence. Within recent years, dozens of recalls because of *L. monocytogenes* in ready-to-eat poultry products have made the news, some having wide-ranging multistate and international involvement (27,28,50,56,63).

The source of *L. monocytogenes* contamination of turkey processing plants and products has not been determined; however, it is widely believed that *L. monocytogenes* is a food safety problem because of its wide presence in the environment and the inability to properly sanitize processing equipment and workers' hands and gloves, rather than any intrinsic contamination of the bird (12,13,36,54,55,60). However, a number of studies have implicated the colonization of incoming birds with subsequent contamination of the processing environment and both raw and cooked product (6,7,43,61). Based on our experience studying chronic bone and joint infection in turkeys (35), we have hypothesized that *L. monocytogenes* contamination of turkey products and processing plants may be, in part, due to the endogenous contamination of chronically infected birds that harbor bacteria within the joints, bone, and soft tissues (33,34). Besides being a widely dispersed bacterium that can survive in most environments, *L. monocytogenes* is also a facultative, intracellular pathogen that can persist and multiply in cells of the monocyte-macrophage system (26,41), as well as in enterocytes, hepatocytes, and various protozoa (11,47). *Listeria monocytogenes* is known to infect joints, tendons, and bone

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(8,21,32,37,49) and is, on occasion, associated with abscesses in animal infections (29). A number of studies have reported that the incidence of *L. monocytogenes* contamination within poultry plants increases as the birds pass through the plant, with the greatest contamination occurring in the latter stages of processing (12,24,25,54,55,64). In one study (24), contamination on the hands and gloves of poultry handlers increased from 20% after chilling, to 45.5% after cutting carcasses, and to 59% when the parts were packaged. This observation supports the position that endogenous bacteria within the tissues of normal-appearing birds may be an overlooked source of processing-plant and product contamination when turkeys are cut up for further processing.

In a previously reported challenge study (33), we established that day-of-age air-sac injection of turkey poults with the Scott A strain of *L. monocytogenes* results in respiratory disease, septicemia, high levels of mortality, bursal atrophy, and bacterial colonization of organs and joints. The objectives of the present study were to compare less-invasive and more natural challenge routes (oral and oculonasal) for their ability to produce disease in day-of-age turkey poults under differing housing conditions.

MATERIALS AND METHODS

Experimental design. One-day-old, male turkey poults ($n = 280$) were obtained from a commercial hatchery and placed in either 3.9-m^2 floor pens on fresh pine shavings or in 0.336-m^2 Petersime battery-brooder cages (Petersime Incubator Co., Gettysburg, OH) that were lined with plastic-coated paper and placed in the same room as the floor pens. Two separate, biosecure buildings were used, with nonchallenged birds housed separately but under identical environmental conditions, and were maintained using strict biosecurity. Birds in each housing type were divided into duplicated challenge groups and challenged at 1 day of age as follows: unchallenged controls, oral challenge, or oculonasal challenge. Birds were raised under incandescent lighting with a 23-hr day and 1-hr night schedule and were provided with *ad libitum* access to water and a standard turkey starter diet that met or exceeded the recommendations of the National Research Council (52). At 7 days of age, birds challenged in batteries were transferred to floor pens. All animal research procedures were evaluated and approved by the Institutional Animal Care and Use Committee of the University of Arkansas.

Listeria monocytogenes. The serotype 4b strain used in this study, designated Scott A, is a human-epidemic isolate that was obtained from the U.S. Food and Drug Administration, Bacterial Physiology Branch (Cincinnati, OH). An early log-phase inoculum was prepared by adding two loopfuls of an overnight culture on blood agar to 100 ml of tryptose phosphate broth (TPB) and incubating for 2.5 hr in a 37 C shaking-water bath. The culture was held overnight at 4 C, while a standard plate count was made. Before inoculation of birds, the suspension was adjusted to yield 1×10^7 CFU/ml.

Oral challenge. There were 50 orally gavaged birds housed in 4 floor pens and 50 housed in 4 battery brooder cages. Twelve or thirteen birds in each duplicated pen were each challenged with 1 ml of an approximately 10^7 CFU/ml inoculum of *L. monocytogenes* in TPB.

Oculonasal challenge. There were 50 oculonasally challenged birds housed in four floor pens and 50 housed in four battery brooder cages. In each duplicated pen, 12 or 13 birds were challenged with 200 µl of the same 10^7 CFU/ml inoculum of *L. monocytogenes*. Birds were inoculated with a 50-µl drop of culture in each eye and nostril (200 µl/bird) using a tuberculin syringe; thus, the challenge was 2×10^6 CFU/bird.

Nonchallenged controls. There were four pens and four battery cages containing 10 birds each of nonchallenged controls, which were housed in a separate, biosecure building.

Necropsy. Morbidity and mortality were monitored for 2 wk postchallenge. Birds were observed for clinical signs of infection, including depression, reluctance to walk, and neurologic signs, such as torticollis. Each dead bird was scored for increase in gall bladder size using a scale of 0, 1, or 2. Lesions were defined as an increase in size and the presence of congealed bile. Liver, heart, spleen, and bursa of Fabricius were excised and weighed. The liver, brain, and knee synovial tissues of every bird were cultured with sterile transport swabs and directly plated onto University of Vermont modified *Listeria* enrichment media (UVM, Difco Laboratories, Detroit, MI), containing *Listeria* selective supplement (SR140E, Oxoid Limited, Ogdensburg, NY), moxalactam antimicrobial supplement (Becton Dickenson, Cockeysville, MD), and ferric ammonium citrate (FAC, Sigma-Aldrich Corp. St. Louis, MO). Isolated colonies were identified using Gram staining, hemolysis on Columbia blood agar (Remel, Microbiology Products, Lenexa, KS), biochemical tests (API *Listeria* Kit, Bio-Mérieux Vitek Inc., Hazelwood, MO), and the BioLog Microbial ID system (Biolog, Inc., Hayward, CA).

On day 7 postchallenge, three birds from each control floor pen ($n = 12$) and five birds from each challenge floor pen ($n = 20$ for each challenge) were bled, necropsied, and cultured for *L. monocytogenes* as described. Birds were bled by cardiac puncture and blood was placed into ethylenediaminetetraacetic acid (EDTA)–coated glass tubes. Total leukocyte white blood cell (WBC) counts and the numbers and percentages of heterophils (Het), lymphocytes (Lym), monocytes (Mono), eosinophils (Eos), basophils (Baso), and platelets (Plt) were determined using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL), which employs both electronic impedance and laser light scattering and has been standardized for analysis of turkey blood.

Statistics. All percentage data were subjected to arcsine transformation. Pen was used as the experimental unit, and means were analyzed using the general linear models procedure of SAS software (62). Significant differences between treatments were separated using the least-square means procedure. Unless otherwise stated, a P value of ≤ 0.05 was considered significant.

RESULTS

Challenge of turkeys with *L. monocytogenes* by the oculonasal route resulted in higher main-effect mean percentage of mortality as compared with nonchallenged controls and tended to be higher than the oral route ($P = 0.05$; Table 1). However, this difference in mortality was only seen in challenged birds that were confined in battery cages for the first week after challenge ($P = 0.002$; Table 1). Birds died suddenly with no clinical signs of infection observed.

Week 1 and week 2 body weights were significantly decreased in the oculonasal challenge group as compared with both unchallenged controls and oral challenge ($P = 0.0001$; Tables 2 and 3). This decrease occurred in both floor pens and battery cages. Week 1 body weights were significantly higher in birds raised in battery cages as compared with floor pens for all challenge groups ($P = 0.0001$). Week 2 body weights were significantly higher in birds housed in battery cages for the first week only in nonchallenged control and orally challenged treatment groups (Table 3). There was a significant interaction between treatment and housing for body weight during both weeks 1 and 2 ($P = 0.02$; Tables 2 and 3).

The main effect mean gall bladder lesion score was increased after challenge ($P < 0.0001$) and housing in battery cages ($P = 0.0002$; Table 4). The relative weights of liver, heart, and spleen (organ weight/body weight) were significantly higher in both orally and oculonasally challenged birds that were necropsied 7 days postinfection compared with nonchallenged controls, and there was a tendency for higher relative weights of the bursa of Fabricius in both challenged groups ($P = 0.06$; Table 5).

The absolute number and the percentage of Lym were higher in birds challenged by the oculonasal route and bled 7 days

Table 1. Percentage of mortality of turkeys raised in floor pens or placed in paper lined battery cages for the first week and exposed to oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[A]

Treatment	% Mortality		Treatment main effect mean ($P = 0.05$)
	Floor pens	Battery cages	
Control	0 ± 0	7 ± 3^{b}	4^{a} ($n = 79$)
Oral Lm[B]	4 ± 2	6 ± 2^{b}	5^{ab} ($n = 100$)
Oculonasal Lm[C]	2 ± 1^{y}	20 ± 4^{ax}	11^{b} ($n = 100$)
Housing main effect mean ($P = 0.002$)	Floor pens	2^{b} ($n = 138$)	Treatment × housing interaction ($P = 0.07$)
	Battery cages	11^{a} ($n = 141$)	

[A]Data represent the mean and SEM.
[B]Oral inoculum consisted of 1 ml of a 1×10^{7} CFU/ml culture (1×10^{7} CFU/bird).
[C]Oculonasal inoculum consisted of a 50-µl drop of the same culture in each eye and nostril (2×10^{6} CFU/bird).
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

Table 2. Week 1 body weight (g) of turkeys raised either in floor pens or in paper-lined battery cages for the first week and exposed to oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[A]

Treatment	Body weight (g)		Treatment main effect mean ($P < 0.0001$)
	Floor pens	Battery cages	
Control	110.0 ± 19^{ay}	137.1 ± 23^{ax}	123.6^{a} ($n = 79$)
Oral Lm[B]	115.0 ± 17^{ay}	131.0 ± 17^{ax}	123.0^{a} ($n = 100$)
Oculonasal Lm[C]	99.5 ± 17^{by}	118.0 ± 21^{bx}	108.8^{b} ($n = 100$)
Housing main effect mean ($P < 0.0001$)	Floor pens	108.0^{b} ($n = 138$)	Treatment × housing interaction ($P = 0.02$)
	Battery cages	128.1^{a} ($n = 141$)	

[A]Data represent the mean and SEM.
[B]Oral inoculum consisted of 1 ml of a 1×10^{7} CFU/ml culture (1×10^{7}CFU/bird).
[C]Oculonasal inoculum consisted of a 50-µl drop of the same culture in each eye and nostril (2×106 CFU/bird).
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

Table 3. Week 2 body weight (g) of turkeys raised either in floor pens or in paper-lined battery cages for the first week and exposed to oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[A]

Treatment	Body weight (g)		Treatment main effect mean ($P < 0.0001$)
	Floor pens	Battery cages	
Control	267.4 ± 69^{aby}	333.1 ± 71^{ax}	305.6^{a} ($n = 67$)
Oral Lm[B]	277.0 ± 65^{ay}	311.7 ± 69^{ax}	298.7^{a} ($n = 80$)
Oculonasal Lm[C]	232.5 ± 54^{b}	254.0 ± 84^{b}	245.9^{b} ($n = 77$)
Housing main effect mean ($P < 0.0001$)	Floor pens	259.1^{b} ($n = 87$)	Treatment × housing interaction ($P = 0.02$)
	Battery cages	299.7^{a} ($n = 137$)	

[A]Data represent the mean and SEM.
[B]Oral inoculum consisted of 1 ml of a 1×10^{7} CFU/ml culture (1×10^{7}CFU/bird).
[C]Oculonasal inoculum consisted of a 50-µl drop of the same culture in each eye and nostril (2×106 CFU/bird).
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

Table 4. Gall bladder lesion scores of mortalities and of necropsied birds. Turkeys were raised either in floor pens or in paper-lined battery cages for the first week and were exposed to oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[AB]

Treatment	Mean lesion score		Treatment main effect mean ($P < 0.0001$)
	Floor pens	Battery cages	
Control	0 ± 0^{b}	0.33 ± 0.33^{b}	0.07^{b} ($n = 15$)
Oral Lm[C]	0.63 ± 1.14^{ay}	1.33 ± 0.33^{ax}	0.72^{a} ($n = 25$)
Oculonasal Lm[D]	0.83 ± 0.15^{ay}	1.40 ± 0.16^{ax}	1.04^{a} ($n = 28$)
Housing main effect mean ($P = 0.0002$)	Floor pens	0.56^{b} ($n = 16$)	Treatment × housing interaction ($P = 0.76$)
	Battery cages	1.19^{a} ($n = 52$)	

[A]Gall bladders were scored for size and presence of congealed bile on a scale of 0–3.
[B]Data represent the mean and SEM.
[C]Oral inoculum consisted of 1 ml of a 1×10^{7} CFU/ml culture (1×10^{7}CFU/bird).
[D]Oculonasal inoculum consisted of a 50-µl drop of the same culture in each eye and nostril (2×106 CFU/bird).
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

Table 5. Relative organ weights (organ weight/body weight) of turkeys necropsied at 7 days after oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[A]

	Control ($n = 12$)	Oral Lm[B] ($n = 20$)	Oculonasal Lm[C] ($n = 20$)	P value
BWT[D]	114.4 ± 4.0[a]	115.6 ± 3.8[a]	102.1 ± 3.8[b]	0.05
Liver	3.39 ± 0.11[b]	3.69 ± 0.14[a]	3.94 ± 0.18[a]	0.02
Heart	0.57 ± 0.02[b]	0.70 ± 0.02[a]	0.69 ± 0.02[a]	0.0006
Bursa	0.15 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.06
Spleen	0.05 ± 0.001[b]	0.06 ± 0.001[a]	0.06 ± 001[a]	0.05

[A]Data represent the mean and SEM of organ weights/body weight.
[B]Oral inoculum consisted of 1 ml of a 1×10^7 CFU/ml culture (1×10^7CFU/bird).
[C]Oculonasal inoculum consisted of a 50-μl drop of the same culture in each eye and nostril (2×106 CFU/bird).
[D]BWT = body weight.
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

postinfection as compared with both nonchallenged and orally challenged birds (Table 6). There were no significant differences in total WBC counts or in numbers or percentages of Het, Lym, Mono, Baso, or Eos (Table 6).

Listeria monocytogenes was isolated by direct plating on modified *Listeria* enrichment UVM from the gall bladder, brain, and knee synovial tissue of one bird that died 3 days postinfection and that had been challenged by the oculonasal route and housed in a battery cage. The biochemical test results and Biolog profile of these three isolates were identical to the Scott A challenge strain (results not shown).

DISCUSSION

Under the conditions of this study, it is impossible to exclude either oral or respiratory exposure from each of the challenges. However, these data strongly suggest that an oculonasal challenge of 1-day-old turkey poults with *L. monocytogenes* Scott A is more pathogenic than is a five times higher dosage when using an oral challenge. Because human illness with *L. monocytogenes* is primarily associated with the consumption of contaminated food, the oral route of infection is generally thought to be the most important and relevant for study; and thus, most contemporary animal studies use the oral route. However, the respiratory system is an important entry point for bacterial pathogens during confined poultry production on litter and has been shown to be important in studies of *L. monocytogenes* virulence in mammals (23,39,59). Respiratory challenge was shown to be the most consistent route in experimental infection of mice, hamsters, guinea pigs, rabbits, rhesus monkeys, and lambs with *L. monocytogenes* (39). These authors suggested that in nature *L. monocytogenes* infection most likely occurs through the respiratory tract and associated mucosal surfaces and conjunctiva. Respiratory challenge with *L. monocytogenes* has become a standard model for investigating pulmonary host-defense mechanisms and host-pathogen interactions in rats and mice (1,40,44,45,51).

Previous reports of oral and contact *L. monocytogenes* exposure of chickens (3,4) and intra-abdominal challenge of turkeys (48) indicate that young birds are far more susceptible to challenge with this organism than are older birds. This has also been true in our experience because oral or respiratory challenge of 5-wk-old turkeys with *L. monocytogenes* Scott A results in minimal morbidity and mortality (34). Respiratory exposure to dust and pathogens in litter is a major cause of disease and condemnation in poultry production, and the respiratory route of infection is increasingly important as producers rely on built-up litter to defray the costs of both litter and litter disposal. (5). Although some surveys have failed to find *L. monocytogenes* in the litter of turkey houses (55) or broiler houses (46,54), litter contamination with *L. monocytogenes* has also been reported to be a persistent problem (15,16,17). Because *L. monocytogenes* is commonly found in fecal material and on plant materials, as well as in soil and water, and because it can survive for long periods under adverse conditions (22), it might be expected to have a sporadic presence in poultry litter and dust. In a discussion of listeriosis in poultry production (57), it was suggested that "it is impractical to attempt to monitor the *Listeria*-free status of poultry

Table 6. Total leukocyte (WBC), and lymphocyte (Lym) counts and WBC percentages of Lym, heterophils (Het), monocytes (Mono), basophils (Baso), and eosinophils (Eos) of turkeys bled and necropsied at 1 wk after oral or oculonasal challenge with *Listeria monocytogenes* (Lm) at 1 day of age.[A]

	Control ($n = 11$)	Oral Lm[B] ($n = 20$)	Oculonasal Lm[C] ($n = 19$)	P value
WBC[D]	10.3 ± 0.9	12.1 ± 1.1	12.7 ± 1.3	NS[E]
Lym[D]	0.8 ± 0.2[b]	1.1 ± 0.2[b]	2.6 ± 0.6[a]	0.01
% Het	67.9 ± 3.9	66.9 ± 2.8	59.4 ± 0.3.7	NS
% Lym	7.8 ± 1.4[b]	8.2 ± 1.2[b]	18.6 ± 0.2.9[a]	0.0006
% Mono	23.8 ± 3.5	24.2 ± 2.7	21.3 ± 2.5	NS
% Baso	0.30 ± 0.09	0.44 ± 0.11	0.45 ± 0.16	NS
% Eos	0.16 ± 0.15	0.17 ± 0.04	0.21 ± 0.08	NS

[A]Data represent the mean and SEM.
[B]Oral inoculum consisted of 1 ml of a 1×10^7 CFU/ml culture (1×10^7CFU/bird).
[C]Oculonasal inoculum consisted of a 50-μl drop of the same culture in each eye and nostril (2×106 CFU/bird).
[D]Data represent cell number × 1000/μl of peripheral blood.
[E]NS = not significant.
[ab]Means within a column with no common superscript differ significantly ($P \leq 0.05$).
[xy]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

flocks because *Listeria* are widespread in nature and are in the alimentary tract of up to 50% of clinically healthy birds.''

The infection due to oculonasal challenge, seen in the present study, was characterized by lack of clinical signs, with sudden mortality, decreased body weight, and relative weight of liver, heart, and spleen (Table 5) and with increases in gall bladder lesion scores and lymphocyte numbers and percentages (Tables 4 and 6). The gross gall bladder lesions seen in both orally and oculonasally challenged turkeys may be of epidemiologic significance because biliary replication and excretion has been reported in mice and is hypothesized to be involved in amplification and dissemination of the organism into the environment (30,31).

In this study, the paper liners used in the battery cages for the first week, as well as the smaller surface area in the battery, would have increased contact with the bacterial challenge, particularly if it were amplified in the gall bladder and passed in the feces, and would also have kept the bacteria hydrated. Reports of *L. monocytogenes* outbreaks associated with wet litter conditions and environmental stress (10,42) suggest that, under the right circumstances, *L. monocytogenes* associated with wet litter can cause clinical disease in poultry. The paradoxical increase in body weight seen in the battery-housed birds may have been due to the confined nature of the first week of brooding with easier access to feed and less room for movement.

For unknown reasons, *L. monocytogenes* serotype 4b has been the major serotype associated with human epidemics in both the United States and in Europe (14,20,38,50,67,68). The serotype 4b strain used in this study, designated Scott A, is a human-epidemic isolate that was previously used to orally challenge 2-day-old chickens, resulting in 18% mortality (36). In that study, most of the chickens eliminated the organism within 9 days, whereas long-term (28 days) infection was maintained in 1 of 10 challenged chicks. Oral challenge in the present study did not significantly increase mortality or affect body weight or lymphocytosis, but it did result in an increase in gall bladder lesion score and changes in relative organ weights. A serotype 4b isolate was also reported to cause encephalitis in broiler chickens (9). In that case report, the organism was very difficult to isolate, and successful culture was only possible directly from the brain stem.

In various studies *L. monocytogenes* has been isolated from up to 33% of healthy poultry sampled (66). Once isolated, *L. monocytogenes* grows well on many bacteriologic media; however, its initial isolation from naturally and experimentally infected animal tissues has been described as being very difficult, often involving months of refrigeration and repeated isolation attempts (18). It was reported that most orally exposed animals do not develop clinical signs of infection but may harbor the bacteria for long periods in an unculturable form, suggesting that animals, including poultry, can be asymptomatic carriers of this organism (29). The mechanisms by which cold enrichment enhances recovery are still not understood, but it has been suggested that, in clinical specimens, *L. monocytogenes* may be present within phagocytic cells, and cold storage facilitates their release (66). Recently, our laboratory has reported the isolation of *L. monocytogenes* from the livers and knee synovial tissues of up to 50% of stressed and environmentally challenged turkeys using pre-enrichment in UVM-1 medium and Fraser broth and real-time polymerase chain reaction detection (19).

However, in the present study, only direct plating of culture swabs on *Listeria*-selective medium was used for bacterial isolation, and the challenge Scott A strain was isolated from the gall bladder, brain, and knee joint of only one dead poult. Although this low rate of isolation does not support the hypothesis that such chronic and unapparent infections are important in processing plant and product contamination, higher isolation rates may have been obtained using pre-enrichment. A low level of intrinsic bacterial carriage could still be responsible for the sporadic seeding of processing plants with pathogenic and persistent strains of bacteria. Bacteria that colonize bones and joints possess the biofilm phenotype and are known to be notoriously difficult to culture (2,53,65). The biofilm phenotype has also been associated with *L. monocytogenes* persistence in processing plants (58), suggesting that the stress of surviving the host immune response during chronic infection may predispose *L. monocytogenes* to a more persistent phenotype. Further study is needed to develop better methods for the isolation and quantitation of *L. monocytogenes* from animal tissues.

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